Putrescine acetyltransferase (PAT/SAT1) dependent GABA synthesis in astrocytes

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Abstract

GABA synthesis in astrocytes mediates tonic inhibition to regulate patho-physiological processes in various brain regions. Monoamine oxidase B (MAO-B) has been known to be the most important metabolic enzyme for synthesizing GABA from the putrescine degradation pathway. MAO-B converts $N^1$-acetylputrescine to $N^1$-acetyl-$\gamma$-aminobutyraldehyde and hydrogen peroxide ($H_2O_2$). Putrescine acetyltransferase (PAT), also known as spermidine and spermine $N^1$-acetyltransferase 1 (SAT1), has been thought to be a feasible candidate enzyme for converting putrescine to $N^1$-acetylputrescine. However, it has not been rigorously investigated or determined whether PAT/SAT1 contributes to GABA synthesis in astrocytes. To investigate the contribution of PAT/SAT1 to GABA synthesis in astrocytes, we conducted sniffer patch and whole-cell patch experiments with gene silencing of PAT/SAT1 by $Sat1$ shRNA expression. Our results showed that the gene silencing of PAT/SAT1 significantly decreased the MAO-B-dependent GABA synthesis, which was induced by putrescine incubation, leading to decreased $Ca^{2+}$-dependent release of GABA in vitro. Additionally, we found that, from the brain slice ex vivo, putrescine incubation induces tonic GABA inhibition in dentate gyrus granule cells, which can be inhibited by MAO-B inhibitor, selegiline. Consistent with our in vitro results, astrocytic gene silencing of PAT/SAT1 significantly reduced putrescine incubation-induced tonic GABA current, possibly by converting putrescine to $N^1$-acetylputrescine, a substrate of MAO-B. Our findings emphasize a crucial role of PAT/SAT1 in MAO-B-dependent GABA synthesis in astrocytes.
Introduction

$\gamma$-aminobutyric acid (GABA) is a crucial amino acid and one of the primary neurotransmitters in the central nervous system (CNS). Dysregulated GABA signaling has been linked to a range of brain diseases, such as depression, schizophrenia, and seizures (Kaloueff and Nutt, 2007; Marques et al., 2021; Treiman, 2001). Tonic inhibition is one of the ways in which GABA$\text{A}$ receptor-mediated inhibition occurs (Farrant and Nusser, 2005). In tonic inhibition, which is different from phasic inhibition, ambient GABA persistently activates extrasynaptic GABA$\text{A}$ receptors, inducing GABA-mediated tonic conductance. Tonic activation of GABA$\text{A}$ receptors regulates neuronal excitability, maintaining the balance between excitation and inhibition and filtering out irrelevant information. We have previously reported that astrocyte release GABA through Bestrophin1 to mediate tonic inhibition in diverse brain regions, including the cerebellum, hippocampus, thalamus, and substantia nigra pars compacta (Heo et al., 2020; Kwak et al., 2020; Lee et al., 2010). Astrocytes actively participate in signal transmission through tonic inhibition, highlighting their crucial role in the regulation of brain function. Thus, identifying the enzymes for GABA synthesis in astrocytes is critical for understanding role of astrocytes in the brain function.

GABA synthesis in astrocytes is different from neuronal GABA synthesis. In the neuron, glutamate is the only source for GABA synthesis, and decarboxylation by glutamic acid decarboxylase (GAD) produces GABA. In astrocytes, putrescine is another source of GABA synthesis. We previously have shown that increased putrescine induces GABA synthesis in astrocytes. Increased putrescine levels in the hippocampal astrocytes have been observed in Alzheimer's disease, and elevated putrescine levels increase GABA synthesis in astrocytes and tonic GABA current in the hippocampus (Ju et al., 2022). There are two ways of putrescine to GABA synthesis in astrocytes, diamine oxidase (DAO)-dependent and MAO-B-dependent pathways (Kwak et al., 2020). In the DAO-dependent GABA synthesis, putrescine is directly oxidized to $\gamma$-aminobutyraldehyde by DAO, and then $\gamma$-aminobutyraldehyde is converted to GABA by aldehyde dehydrogenase 1 family, member A1 (ALDH1A1). In contrast to the DAO-dependent pathway, there are four steps in the MAO-B-dependent pathway. N$^1$-acetylputrescine converted from putrescine is oxidized by MAO-B and becomes N$^1$-acetyl-$\gamma$-aminobutyraldehyde. N$^1$-acetyl-$\gamma$-aminobutyraldehyde is dehydrogenated to N$^1$-acetyl-GABA by ALDH1A1 and further deacetylated to GABA by sirtuin 2 (Bhalla et al., 2023). Every step except the first step has been defined in the astrocytic MAO-B-dependent GABA synthesis. Although PAT/SAT1 has been proposed as the enzyme for putrescine to N$^1$-acetylputrescine conversion, PAT/SAT1’s role in GABA synthesis of astrocytes has not been rigorously investigated yet (Petroff, 2002; Yoon and Lee, 2014).

In this study, we hypothesized that PAT/SAT1, well-known as polyamine acetyltransferase, is the astrocytic enzyme involved in putrescine-to-GABA synthesis. To investigate the role of PAT/SAT1 in astrocytic GABA synthesis, we conducted immunocytochemistry to measure putrescine-induced GABA levels after silencing PAT/SAT1 expression in cultured hippocampal astrocytes. We also performed two-cell sniffer patch experiments to examine GABA synthesis and release from cultured astrocytes. Furthermore, we established an *ex vivo* model of putrescine incubation to induce tonic GABA current. Using this model, we measured tonic GABA currents induced by putrescine incubation and confirmed our findings in brain slices by silencing the astrocyte-specific
gene for PAT/SAT1. Our results demonstrate that PAT/SAT1 is involved in the putrescine-to-GABA synthesis pathway in astrocytes.
Materials and Methods

Animals

All C57BL/6 mice were group-housed in a temperature- and humidity-controlled environment with a 12 h light/dark cycle and had free access to food and water. All animal care and handling were approved by the Institutional Animal Care and Use Committee of the Institute for Basic Science (IBS-2020-005; Daejeon, Korea). For the slice patch, 8- to 20-week-old male C57BL/6 mice were used.

Methods

Cell culture

Primary hippocampal astrocytes were prepared from 1-day postnatal C57BL/6 mice as previously described (Woo et al., 2012). The cerebral cortex was dissected free of adherent meninges, minced, and dissociated into a single-cell suspension by trituration. Dissociated cells were plated onto plates coated with 0.1 mg/ml poly-D-lysine (Sigma). Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM, Corning) supplemented with 4.5 g/L glucose, L-glutamine, sodium pyruvate, 10 % heat-inactivated horse serum, 10 % heat-inactivated fetal bovine serum, and 1000 units/ml of penicillin–streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5 % CO2. Three days later, cells were vigorously washed with repeated pipetting using medium and the media was replaced to remove debris and other floating cell types.

Quantitative real-time RT-PCR

Quantitative real-time RT-PCR was carried out using SYBR Green PCR Master Mix as described previously (Kwak et al., 2020). Briefly, reactions were performed in duplicates in a total volume of 10 ml containing 10 pM primer, 4 ml cDNA, and 5 ml power SYBR Green PCR Master Mix (Applied Biosystems). The mRNA level of each gene was normalized to that of Gapdh mRNA. Fold-change was calculated using the 2ΔΔCT method. The following sequences of primers were used for real-time RT-PCR. Gapdh forward: 5` - ACC CAG AAG ACT GTG GAT GG -3`; Gapdh reverse: 5` -CAC ATT GGG GGT AGG AAC AC-3`; Sat1 forward: 5` - GAC CCC TGA AGG ACA TAG CA -3`; Sat1 reverse: 5` - CCG AAG CAC CTC TTC TTT TG-3`.

Sniffer patch from primary cultured astrocytes

Three days before, Sat1 shRNA was transfected into primary hippocampal astrocytes. On the day of sniffer patch, HEK 293T cells expressing GABAc sensor were dissociated, triturated, added onto the cover glass with cultured astrocytes, and then allowed to settle for at least 1 h before sniffer patching. After HEK cells settled, cultured astrocytes were incubated with 5 μM Fura-2AM (mixed with 1ml of external solution containing 5 ml of 20 % pluronic acid, Invitrogen) for 40 min and washed at room temperature and subsequently transferred to a
microscope stage. External solution contained 150 mM NaCl, 10 mM HEPES, 3 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, 5.5 mM glucose, pH adjusted to pH 7.3 and osmolality to 325 mOsmolkg⁻¹. Intensity images of 510-nm wavelength were taken at 340-nm and 380-nm excitation wavelengths using iXon EMCCD (DV887 DCS-BV, ANDOR technology). The two resulting images were used for ratio calculations in Axon Imaging Workbench version 11.3 (Axon Instruments). To induce Ca²⁺-dependent astrocyte gliotransmission by activating PAR1 and TRPA1 receptors, respectively, TFLLR was either locally puffed (Kwak et al., 2020) or poked with a glass pipette (Oh et al., 2019) as previously. GABAc-mediated currents were recorded from HEK 293T cells under voltage clamp (Vh = -60 mV) using Axopatch 200A amplifier (Axon Instruments), acquired with pClamp 11.3. Recording electrodes (4–7 MΩ) were filled with 110 mM Cs-glucuronate, 30 mM CsCl, 0.5 mM CaCl₂, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na₃GTP and 10 mM BAPTA (pH adjusted to 7.3 with CsOH and osmolality adjusted to 290–310 mOsmkg⁻¹ with sucrose). For simultaneous recordings, Imaging Workbench was synchronized with pClamp 11.3. To normalize differences in GABAc receptor-expression on the HEK 293T cells, 100 μM of GABA in the bath was applied to maximally activate the GABAc receptors after current recording. Normalization was then accomplished by dividing the current induced by GABA released from astrocytes by the current induced by bath application of GABA.

**Immunocytochemistry of cultured hippocampal astrocytes**

Cultured primary astrocytes were fixed in 4 % PFA in PBS for 15 mins and incubated for 1.5 h in a blocking solution (0.3 % Triton-X, 4 % normal serum in 0.1 M PBS) and then immunostained with a mixture of primary antibodies [Guineapig anti-GABA; AB175 (1:200) and chicken anti-GFAP; AB5541 (1:500)] in a blocking solution at 4 °C on a shaker overnight. After washing in PBS 3 times, samples were incubated with corresponding fluorescent secondary antibodies [Donkey anti-guineapig alexa 488; 706-475-148 (1:200) and donkey anti-chicken alexa 405; 703-475-155 (1:200)] for 2 h and then washed with PBS 3 times. Finally, samples were mounted with fluorescent mounting medium (Dako) and dried. A series of fluorescent images were obtained with a Zeiss LSM900 confocal microscope and processed for further analysis using ImageJ program. Any alterations in brightness or contrast were equally applied to the entire image set. Specificity of primary antibody and immunoreaction was confirmed by omitting primary antibodies or changing fluorescent probes of the secondary antibodies.

**Virus injection**

Mice were anesthetized with vaporized isoflurane and placed into stereotaxic frames (Kopf). The scalp was incised, and a hole was drilled into the skull above the dentate gyrus (anterior/posterior, -1.5mm; medial/ lateral, -1.2 or +1.2mm from bregma, dorsal/ventral, -1.8mm from the brain surface). The virus was loaded into a glass needle and injected bilaterally into the dentate gyrus at a rate of 0.2 μl min⁻¹ for 5 min (1 μl per each site) using a syringe pump (KD Scientific). Virus was generated from Institute for Basic science virus facility (IBS virus facility). AAV-GFAP-mCh, Lenti-pSico-Scrambled shRNA-GFP, and Lenti-pSico-PAT/SAT1 shRNA-GFP
viruses were used in each experiment. Mice were used for patch-clamp 3 weeks after the virus injection.

**Preparation of brain slices**

Mice were anesthetized with isoflurane and decapitated to remove the brain. The brains were sectioned in ice-cold slicing solution (234 mM sucrose, 2.5 mM KCl, 104 mM MgSO₄, 1.25 mM NaH₂PO₄, 24 mM NaHCO₃, 0.5 mM CaCl₂·2H₂O and 11 mM glucose). Horizontal slices (300 μm thick) were prepared with a vibrating-knife microtome Linear Slicer Pro7 (D.S.K, Japan). For stabilization, slices were incubated in room temperature for at least 1 h in a solution containing 124 mM NaCl, 3 mM KCl, 6.5 mM MgSO₄, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, 1 mM CaCl₂·2H₂O and 10 mM glucose, and simultaneously equilibrated with 95% O₂/5% CO₂ at 25 °C. In some experiments, slices were incubated with blockers during stabilization for at least 2 h and slices were incubated with putrescine and selegiline one hour before recording.

**Tonic GABA recording**

Prepared slices were transferred to a recording chamber that was continuously perfused with ACSF solution (flow rate, 2 ml min⁻¹). The slice chamber was mounted on the stage of an upright Olympus microscope and viewed with a 63x water immersion objective (0.90 numerical aperture) with infrared differential interference contrast optics. Cellular morphology was visualized by a charge-coupled device camera and Imaging Workbench software (INDEC BioSystems). Whole-cell recordings were made from granule cell somata located in the DG. The holding potential was -70 mV. Pipettes (resistance 6-8 MΩ) were filled with internal solution [135 mM CsCl, 4 mM NaCl, 0.5 mM CaCl₂, 10 mM HEPES, 5 mM EGTA, 2 mM Mg-ATP, 0.5 mM Na₂-GTP, and 10 mM QX-314, pH adjusted to 7.2 with CsOH (osmolarity, 278 to 285 mOsm/kg⁻¹)]. Baseline current was stabilized with d-AP5 (50 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (20 μM) before measuring tonic current. Electrical signals were digitized and sampled at 50 ms intervals with Digidata 1440 A and a MultiClamp 700B amplifier (Molecular Devices) using pCLAMP10.2 software. Data were filtered at 2 kHz. The amplitude of tonic GABA currents was measured by the baseline shift after bicuculline (100 μM) administration using the Clampfit program. Tonic current was measured from the baseline to bicuculline-treated current. Frequency and amplitude of spontaneous inhibitory postsynaptic currents before bicuculline administration were detected and measured by MiniAnalysis (Synaptosoft).
Results

Gene silencing of PAT/SAT1 decreases GABA synthesis and release in hippocampal astrocytes in vitro

We hypothesized that PAT/SAT1 has a role as the first enzyme in MAO-B-dependent GABA synthesis in astrocytes (Fig. 1A). To investigate this, we expressed Sat1 shRNA in cultured hippocampal astrocytes in vitro to first determine whether Sat1 shRNA reduces Sat1 mRNA expression by quantitative RT-PCR (Fig. 1B). Compared to the control with scrambled shRNA, Sat1 shRNA reduced the expression of Sat1 mRNA by 91.61%, showing that the gene silencing was effective. Next, we investigated whether Sat1 shRNA reduces GABA synthesis and the amount of GABA in hippocampal astrocytes. Hippocampal astrocytes are known to have low amounts of basal putrescine and GABA under physiological conditions (Jo et al., 2014). It is noteworthy that PAT/SAT1 is involved in putrescine generation from spermidine and spermine degradation. To exclude the possibility that gene silencing of PAT/SAT1 decrease putrescine itself and examine the role of PAT/SAT1 in putrescine to GABA synthesis, hippocampal astrocytes were incubated with exogenous 180 μM putrescine. First, intracellular GABA contents in astrocytes were tested by immunocytochemistry. In the scrambled condition, putrescine incubation increased GABA intensity in astrocytes compared to the vehicle control (Fig. 1D, E). However, in the Sat1 shRNA condition, the increase in GABA intensity by putrescine incubation was significantly reduced compared to the scrambled control (Fig. 1D, E). Interestingly, the increase in GABA with putrescine was accompanied by an increase in GFAP intensity (Fig. 1F), supporting the idea that H:O, an additional product of MAO-B-dependent GABA synthesis, may induce astrocytic reactivity (Chun et al., 2020; Chun et al., 2022).

We next attempted to determine if reduced GABA synthesis through gene silencing of PAT/SAT1 would also lead to reduced GABA release from hippocampal astrocytes (Fig. 2A). To measure GABA released by solitary hippocampal astrocytes, we performed the sniffer-patch technique as previously described (Jo et al., 2014; Kwak et al., 2020; Lee and Yoon, 2014) utilizing HEK293T cell that expresses GABAc receptors (referred to as a sensor cell). After seeding the hippocampal astrocyte with the sensor cell, we measured the amount of GABA released by the astrocyte by measuring the whole-cell patch current from the sensor cell (Fig. 2B, E). Given that cytosolic Ca²⁺ is a critical signal for GABA release in astrocytes, and that astrocytic Ca²⁺ can be elevated through either Ca²⁺ release from endoplasmic reticulum (ER) or Ca²⁺ influx from extracellular space, we investigated astrocytic GABA release with two methods to increase astrocytic Ca²⁺. First, to increase astrocytic Ca²⁺ through Ca²⁺ release from ER, TFLLR, a PAR1 agonist, was locally applied to hippocampal astrocyte (Fig. 2B-D). TFLLR application increased astrocytic Ca²⁺ in both scrambled and Sat1 shRNA conditions, but GABA current from sensor cells was significantly reduced in Sat1 shRNA (Fig. 2D), indicating that gene silencing of PAT/SAT1 decreased ER Ca²⁺-dependent GABA release from solitary astrocytes. Next, to increase astrocytic Ca²⁺ through Ca²⁺ influx from extracellular space, mechanical stimulation was delivered with microelectrode poking (Fig. 2E-G), activating astrocytic mechanosensitive channels (e.g. TRPA1) (Oh et al., 2019). Similar to TFLLR, poking stimulation elicited Ca²⁺ responses in both conditions, but GABA current from sensor cells was significantly reduced in Sat1 shRNA (Fig. 2G). Taken together, these results indicate that PAT/SAT1 is
necessary for MAO-B-dependent GABA synthesis and subsequent Ca\(^{2+}\)-dependent GABA release in hippocampal astrocytes in vitro.

**Astrocytic PAT/SAT1 is necessary for tonic GABA inhibition ex vivo**

We showed that putrescine incubation induces GABA synthesis in the primary hippocampal astrocyte culture and GABA synthesis in astrocytes is PAT/SAT1-dependent in the culture system. However, we have not tested whether astrocytic PAT/SAT1 is involved in tonic GABA current in the hippocampus. In the physiological condition, tonic GABA current is not big enough to investigate the necessity of PAT/SAT1 in GABA synthesis. In addition, putrescine level would be reduced under physiological level by gene silencing of PAT/SAT1 which generate putrescine through spermidine and spermine degradation. So, we incubated brain slices with putrescine and conducted a whole-cell patch clamp to test whether putrescine incubation increases tonic GABA current in the hippocampus. To test whether putrescine incubation increases tonic GABA current and whether increased tonic GABA inhibition is dependent on the enzyme MAO-B, we conducted whole-cell patch clamp recordings of tonic GABA inhibition in granule cells of the dentate gyrus. We used the MAO-B inhibitor, selegiline, to ensure the specificity of the observed effects. To minimize variation among slices due to incubation time, we pre-incubated each slice with putrescine and selegiline separately for one hour at room temperature before recording (Fig. 3A). Our results showed that putrescine incubation significantly increased tonic GABA current, an effect that was blocked by selegiline (Fig. 3B, C). We did not observe a significant difference in the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs), but unexpectedly the amplitude of sIPSCs was increased by putrescine incubation. Nevertheless, these findings indicate that putrescine increased tonic GABA inhibition through MAO-B-dependent GABA synthesis.

To investigate whether astrocytic PAT/SAT1 is essential for tonic GABA inhibition induced by putrescine incubation, mice were injected with lentivirus carrying pSico-PAT shRNA-GFP which is Cre-dependent and AAV-GFAP-Cre-mCherry virus into the DG (Fig. 4A). More than 3 weeks after virus injection, we performed whole-cell patch clamp recordings in granule cells of DG (Fig. 4B). We observed that astrocyte-specific PAT/SAT1 gene silencing significantly reduced tonic GABA increased by putrescine incubation (Fig. 4C, D). Therefore, we concluded that astrocytic PAT/SAT1 is necessary for the tonic GABA release induced by putrescine incubation.
Discussion

In this study, we have identified the enzyme responsible for the first step in astrocytic putrescine to GABA synthesis. We observed that putrescine-induced GABA synthesis and release is eliminated by gene silencing of PAT/SAT1 in primary-cultured mouse hippocampal astrocytes, as demonstrated by immunostaining (Fig. 1) and sniffer cell patch (Fig. 2). Moreover, we developed a putrescine incubation model to induce MAO-B-dependent tonic GABA in brain slices, which is typically undetectable under physiological conditions (Fig. 3). Using this newly developed model for tonic GABA induction, we observed that increased tonic GABA is reduced by astrocyte-specific gene silencing of PAT/SAT1 (Fig. 4). These findings demonstrate that PAT/SAT1 is the necessary enzyme for GABA synthesis in astrocytes, and possibly catalyzing the first step of MAO-B dependent GABA synthesis from putrescine. Metabolic analysis should be conducted to confirm the PAT/SAT1 activity of the conversion from putrescine to N\(^{\text{acetyl}}\)putrescine.

PAT/SAT1 has been well known as an acetyltransferase for degradation of polyamine including spermidine and spermine. Polyamines modulate the structure or activity of negatively charged molecules such as nucleic acids and proteins, and polyamine levels are tightly regulated. Dysregulated polyamines have been used as markers for disease, and several studies have focused on polyamine metabolism in astrocytes. Previous research has reported increased polyamine catabolism in astrocytes in various brain diseases and has explained the symptoms of the disease by loss of polyamine function (Merali et al., 2014; Sonninen et al., 2020). However, our results suggest that increased polyamine catabolism results in increased astrocytic MAO-B-dependent GABA synthesis. Our previous reports have shown that astrocytic MAO-B activation causes diverse diseases such as Alzheimer’s disease, Parkinson’s disease, traumatic brain injury and rheumatoid arthritis and symptoms of these diseases are alleviated by pharmacological or genetical inhibition of MAO-B (Chun et al., 2020; Chun et al., 2022; Heo et al., 2020; Jo et al., 2014; Park et al., 2019; Won et al., 2022). Therefore, excessive polyamine catabolism of astrocytes in pathological conditions results in not just loss of polyamine, but also aberrant H\(_2\)O\(_2\) generation, the product of MAO-B-dependent polyamine degradation. Here, we showed that MAO-B-dependent GABA synthesis and release are blocked by astrocyte specific gene silencing of PAT/SAT1. Whether PAT/SAT1 inhibition can alleviate the symptoms of those diseases should be investigated in the future.

The acetylation of putrescine or spermidine and spermine by PAT/SAT1 activity is followed by oxidation through MAO-B or acetylpolypamine oxidase (APAO). During the oxidation of acetylpolypamine, H\(_2\)O\(_2\), one of the reactive oxygen species, is generated (Casero and Pegg, 2009). Our research has shown that astrocytic MAO-B excessively produces H\(_2\)O\(_2\) in pathological conditions (Chun et al., 2020; Chun et al., 2022). This excess H\(_2\)O\(_2\) induces hypertrophy of reactive astrocytes and scar formation near injury sites in traumatic brain injury. Furthermore, H\(_2\)O\(_2\) generated by reactive astrocytes causes pathological symptoms such as tauopathy, neuronal death, and cognitive decline in Alzheimer’s disease, which can be alleviated by MAO-B inhibitor or H\(_2\)O\(_2\) scavenger (Chun et al., 2020). Therefore, inhibition of PAT/SAT1 activity would alleviate H\(_2\)O\(_2\)-derived symptoms by blocking not only MAO-B-dependent but also APAO-dependent H\(_2\)O\(_2\) generation (Fig. 5). It would be interesting to determine the exact contribution of MAO-B and APAO in H\(_2\)O\(_2\) generation in future study.
In addition to reducing harmful molecule, inhibiting PAT/SAT1 would increase spermidine and spermine, which have been shown to have neuroprotective roles in CNS (Frühauf et al., 2015; Ghosh et al., 2020; Liang et al., 2021; Schroeder et al., 2021; Xu et al., 2020; Yatin et al., 2001). There is a report that spermidine intake improves the cognitive function in the physiological conditions of drosophila, mice, and humans (Schroeder et al., 2021). Spermidine and spermine have been shown to improve memory and delay aging in pathological conditions. There are several studies on the neuroprotective function of spermidine and spermine, including their ability to work as radical scavengers, maintain mitochondrial function, and enhance autophagy (Frühauf et al., 2015; Ghosh et al., 2020; Liang et al., 2021; Xu et al., 2020; Yatin et al., 2001). Thus, PAT/SAT1 inhibition could induce accumulation of spermidine and spermine by reducing APAO-dependent degradation, which could ameliorate disease symptoms. Based on these reports, we propose that PAT/SAT1 is a potential therapeutic target for neurodegenerative diseases. Although there is a PAT/SAT1 inhibitor, diminazene aceturate, it is also known to activate angiotensin-converting enzyme 2 (ACE2), which is involved in the function of diverse organs such as the heart, kidney, and lung (Duan et al., 2020). Therefore, a PAT/SAT1-specific inhibitor should be developed to block H2O2 generation and enhance spermidine and spermine levels without causing unintended side effects, enabling investigation of polyamine catabolism in the future.

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References


**Figure 1.** Putrescine acetyltransferase (PAT/SAT1) is a key enzyme in MAO-B-dependent GABA synthesis in hippocampal astrocytes

A. Schematic diagram for MAO-B-dependent GABA synthesis in astrocytes.

B. Representative image of Sat1 shRNA transfected hippocampal astrocytes and Sat1 mRNA level in shRNA transfected hippocampal astrocytes.

C. Experimental timeline of immunocytochemistry.

D. Immunostaining of GABA and GFAP in shRNA transfected hippocampal astrocytes.

E. GABA intensity in GFAP positive area.

F. GFAP intensity.

Data represent mean ± SEM ** p<0.01 (One-way ANOVA).
Figure 2. Knockdown of PAT/SAT1 decreases Ca\textsuperscript{2+}-dependent GABA release from astrocyte in vitro.
A. Experimental timeline for sniffer-patch.
B. Experimental diagram for sniffer-patch of GABA release by PAR1 agonist.
C. Representative traces of sensor current induced by GABA from primary cultured hippocampal astrocytes.
D. Bar graph of sensor current.
E. Experimental diagram for sniffer-patch of GABA release by mechanostimulation.
F. Representative traces of sensor current induced by GABA from primary cultured hippocampal astrocytes.
G. Bar graph of sensor current.
Data represent mean ± SEM ** p<0.01, *** p<0.001 (Student’s t test).
Figure 3. Increased tonic GABA inhibition by putrescine incubation
A. Experimental timeline for tonic GABA recording
B. Representative traces of GABAA receptor-mediated currents recorded from granule cells in the hippocampal slices incubated with putrescine or putrescine + selegiline.
C. Bar graph of tonic GABA current amplitude.
D. Bar graph of sIPSC frequency before bicuculline treatment.
E. Bar graph of sIPSC amplitude before bicuculline treatment.
Data represent mean ± SEM * p<0.05 (One-way ANOVA).
**Figure 4.** Knockdown of PAT/SAT1 decreases GABA release from astrocyte *ex vivo*.
A. Experimental diagram and image of virus injection for astrocytic Sat1 gene silencing.
B. Experimental timeline for tonic GABA recording.
C. Representative traces of GABAA receptor-mediated currents recorded from granule cells in the hippocampal slices incubated with putrescine.
D. Bar graph of tonic GABA current amplitude.
Data represent mean ± SEM ** p<0.01 (Student’s t test).
Figure 5. Schematic diagram of PAT/SAT1’s role in MAO-B-dependent GABA synthesis and APAO-dependent spermidine and spermine degradation.